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PROTOCOL FOR EVALUATING THE NITROGEN STATUS OF LAKE SEDIMENTS

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The approach and methodology to evaluate the nitrogen status of lake sediments, with the ultimate aim of estimating their role as a nitrogen source or sink to the overlying waters, is outlined. The information is derived from five years of research effort ~~by the author and associates~~ on the forms, amounts and transformations of nitrogen in lake sediments. The suggested approach involves monitoring or comparative characterization, or both, of the forms of nitrogen in lake sediments, along with laboratory tests to assess the relative rates of various key nitrogen processes such as nitrification, denitrification, mineralization and immobilization. (EPA)

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PROTOCOL FOR EVALUATING THE NITROGEN STATUS OF LAKE SEDIMENTS

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ABSTRACT

This report outlines the approach, methodology and philosophy needed to undertake evaluation of the nitrogen status of lake sediments, with the ultimate aim of estimating their role as a nitrogen source or sink to the overlying waters. It is gleaned from the knowledge and experience gained from five years of intensive research effort on the forms, amounts and transformations of nitrogen in lake sediments. The sediment environment and sediment nitrogen transformations are reviewed. Suggested approaches to evaluation of the nitrogen status of sediments, along with the required methods, are presented.

The methods are based on those developed or tested in the author's laboratory. The suggested approaches involve monitoring or comparative characterization, or both, of the forms of nitrogen in lake sediments, along with laboratory tests to assess the relative rates of various key nitrogen processes such as nitrification, denitrification, mineralization and immobilization. Suggested additional research needs are included.

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CONCLUSIONS

Lake sediments contain a large reservoir of nitrogen, most of which is in refractory forms and thus not immediately available. The readily available nitrogen of sediments is the ammonium and organic nitrogen present in the interstitial water, and the ammonium nitrogen on the exchange complex. This nitrogen can be released to the overlying water by diffusion and by sediment mixing.

Nitrogen reaches lake sediments by sedimentation, nitrogen fixation, and immobilization of nitrate. The sediment may be regarded as a nitrogen sink because much of this nitrogen is not remineralized to an available form, and because about 60 to 70% of the nitrate-N in sediments is reduced to nitrogen gas. However, sediments also must be regarded as a nitrogen source because sediments contain as much or more available, or potentially available, nitrogen as that added yearly by external sources.

The chemical composition of the Wisconsin lake sediments investigated affected nitrogen transformations and availability. The influence of calcium carbonate appeared to be dominant; calcareous sediments had much higher nitrogen transformation rates than noncalcareous sediments, even though they contained less available nitrogen.

The results of the research effort of this project suggest that it is possible to obtain a set of indices which would indicate the nitrogen status of a lake's sediment.

RECOMMENDATIONS

The indices suggested in this report must be evaluated in a lake sediment survey program. This should be done by agencies with the capability to monitor a wide range and large number of lakes. Such a survey could be coordinated with any existing lake inventory program. Additional funding would be required. However, gaining the capability to predict more accurately the nitrogen status of a lake system should be worth the effort.

The approach should be to obtain the indices suggested over time on a sufficient number of lakes differing in properties and trophic level that statistically significant trends could be ascertained. Then quantitative limits could be placed on the indices which proved to have predictive capability, while those of no benefit could be rejected.

INTRODUCTION

Several recent reviews have pointed out the possible importance of lake sediments as a source or sink of nitrogen to overlying waters[1-9]. However field quantitation of the role of sediments to the nitrogen balance of lakes is extremely difficult, if not impossible, due to the complexity of the required experimental systems and edge effects due to restricted container size. The purpose of this paper is to consider the sediment and lake properties which influence the role of sediments as a source or sink of nitrogen to lakes and outline appropriate analyses which could be undertaken to evaluate the nitrogen status of sediments.

The specific objective of the research project was first to evaluate the forms and amounts of nitrogen compounds in lake sediments, and their relationship to lake trophic status and second, the transformations of nitrogen in sediments. The ultimate objective was to enable prediction of the role of sediments as a nitrogen source or sink to the overlying waters.

THE SEDIMENT ENVIRONMENT

The physical properties of lake sediments vary widely, depending on their composition. At one end of the scale are sandy deposits of relatively high bulk density (ca. 1.5 g/cc) which contain low levels of total and available nitrogen, to low bulk density, (ca. 1.0 to 1.1 g/cc) dispersed deposits containing largely silt and clay-size particulates. A given lake may contain significant amounts of both extremes, with an infinite gradation of sediments with intermediate properties. In many lakes, the finer sized sediments are located in the middle, deeper portions of the lake, due to size sorting during deposition[10]. The composition of the particulate matter of sediments is also highly variable, and may consist of sand, silt, clay (crystalline and amorphous), organic matter and marl (in calcareous sediments) in varying proportions.

The fluidity (i.e., the relative ease of penetration of a sediment by a sampling device or sounding weight)[11] is a parameter of lake sediments that may well be worth estimating as it affects the degree of mixing in sediments. In some lakes, the sediment-water interface is quite diffuse, extending over a range of one to several meters, with gradually increasing bulk density with depth of the sediment below the sediment-water interface. Other lakes have a sharp sediment-water interface, while the majority have a diffuse sediment-water interface zone of a few centimeters[11]. Also, fluidity of the sediment at the sediment-water interface likely will vary widely within the lake, and with time, as sediments are more dispersed under anaerobic than aerobic conditions.

The water content, and related bulk density, of sediments will depend on its fluidity, and also to some extent on the sampling method. Normal water contents of sediments when taken by the Eckman dredge range from 50 to 95% on a volume/volume basis. This water, commonly referred to as interstitial water, contains in general much higher NH_4-N and organic N concentrations than the overlying water. Within the limits of high speed centrifuges, it is all of the same composition, i.e., the NH_4-N content of the water removed by centrifugation at relatively low g-forces is the same as that removed at g-forces to 44,000 X g[12].

The pH of sediments <u>in situ</u> probably does not vary widely and in general is in the slightly acid to neutral range (pH 6-7.5), presumably due to the dominant influence of various redox systems[13]. The lower values are typical of those obtained with softwater Wisconsin sediments while the higher values are representative of hardwater (calcareous) lake sediments. On drying, pH of unbuffered (noncalcareous) sediments can decrease, presumably due to the production of H_2SO_4 on oxidation of sulfides.

The Eh (redox potentials) values of sediments are typically 0 to negative. Graetz et al.[14] obtained data indicating that -250 to -300 mV was the stable Eh value for the sediments they investigated below the sediment-water interface, while *in situ* values of +100 to -100 mV at the sediment-water interface have been measured in Lake Wingra, Wisconsin[15].

SEDIMENT-WATER TRANSFER MECHANISMS

Physical, chemical and biological processes are intimately involved in determining the net uptake or release of nitrogen at the sediment-water interface. These factors are often interrelated, and the overall process of sediment-water interchange is quite complex[8,11]. Because of experimental difficulties, meaningful quantitative field experimental data on nitrogen sediment-water transfer rates are difficult to obtain[2]. Probably the only valid field approach will be observation of the effects of markedly reducing outside nitrogen inputs, or other manipulative schemes such as drawdown, on the nitrogen balance in the lake[11]. However, use of ^{15}N treated sediment in bottles suspended in sediment have shown promise[12,15].

A working model for nitrogen uptake or release by sediments must include the processes that add nitrogen to sediments, control the nitrogen content of the interstitial water, and the release of nitrogen from the interstitial to the overlying waters[8]. A model (Fig. 1) which is currently guiding our thinking involves an active sediment of 5 to 20 cm thickness overlying a historical sediment. The active sediment is being constantly reworked by physical (currents, wave action, fish) and biological (gas formation and ebullition, faunal activity) processes. The historical sediment, on the other hand, is not involved in nutrient exchange with the exception of interaction with seepage water. Recent evidence[11,12] indicates that the 5 to 20 cm depth of mixing in sediments is reasonable for relatively unconsolidated materials.

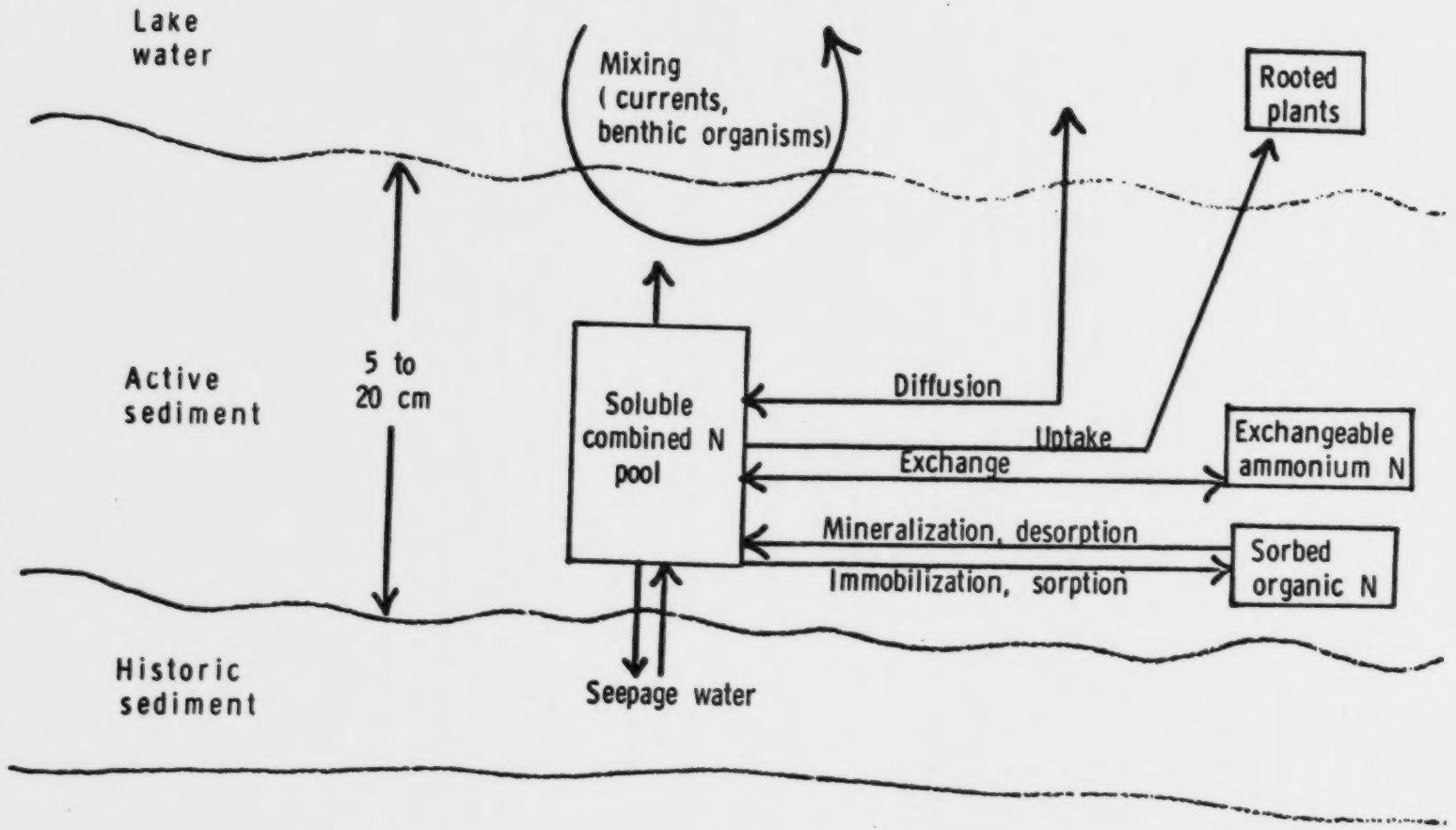


Figure 1. Nitrogen Sediment-Water Interchange Model[8].

FORMS AND AVAILABILITY OF SEDIMENT NITROGEN

A survey of the forms and amounts of nitrogen in surficial and profile samples of sediments from Wisconsin lakes[16,17] has shown that while sediments contain the same forms of nitrogen as do soils, there is a marked difference in the absolute amounts and in many cases, the distribution of nitrogen. As with soils, the nitrogen in lake sediments is present largely as organic-N, and the distribution of the various forms of organic-N determined (total acid hydrolyzable-N, amino acid-N, amino sugar N, hydrolyzable ammonium-N and unidentified-N) was similar to results obtained for organic soils[16]. This work also showed that lake sediments contain significant amounts of NH_4-N entrapped within the lattices of clay minerals, and that sediments from southern Wisconsin lakes contained much greater concentrations of clay-fixed NH_4-N than did northern Wisconsin lake sediments. This was probably related to extent of erosion and soil type in the watersheds of the respective lakes, i.e., the southern Wisconsin lake sediments contain more crystalline clay minerals, eroded from the watershed, than do the northern lake sediments. Fixed NH_4-N is essentially unavailable for microbial uptake[18].

"Available" N in sediments may be divided into two classes, namely: (a) the soluble and exchangeable NH_4-N and soluble organic-N, which is immediately available for transfer to the overlying water; and, (b) that nitrogen potentially available through microbial mineralization of organic-N.

Recent work[19] has shown that soluble- and exchangeable-NH_4-N are equally available to nitrifying bacteria, while Byrnes et al.[12] and Isirimah and Keeney[15] have obtained evidence that sediment NH_4-N is transferred to the overlying water.

An estimate of the potential of sediment organic N to be mineralized is much more difficult to obtain. Austin[20] incubated sediments in carboys at wide water:sediment ratios under aerobic conditions and obtained considerable release. This experiment, however, was so different from actual conditions that valid conclusions cannot be obtained from her work. A more reliable test is to incubate sediments under a narrow water:sediment ratio, anaerobic conditions and cold temperatures (10° C or less) and determine the increase in NH_4-N (soluble and exchangeable) in the sample. Using this approach, Chen et al.[19] found that, with a Lake Mendota sediment, practically no increase in NH_4-N occurred in 14 days under quiescent (non-stirred) conditions but that about 2.1 mg NH_4-N/liter sediment/day was formed when the mixture was stirred. Similar results were obtained for a noncalcareous Trout Lake sediment[21]. Further work in our laboratory[14] has indicated that

sediments have a high potential for producing NH_4-N and releasing this N into the overlying water. This work involved use of simulated lakes in plexiglass columns, incubated under quiescent, anaerobic conditions at room temperature for 48 to 52 days. During this time period, the sediments studied released from 0.38 mg NH_4-N/liter sediment/day (noncalcareous Tomahawk) to 0.65 mg (calcareous Mendota). More recently Isirimah and Keeney[15] estimated a mineralization-release rate of 40 µg NH_4-N/liter sediment/day in Lake Wingra during the summer. These values were obtained in bottles suspended in the sediment, and thus are a conservative estimate. This was further evaluated by field monitoring of the NH_4-N in Lake Wingra sediments[22] where a loss rate of 29 mg NH_4-N/m^2 sediment surface/day was estimated. The actual release rate for calcareous eutrophic Wisconsin lakes thus apparently ranges from about 4 to 29 mg NH_4-N/m^2/day. These estimates show that lake sediments are a significant source of nitrogen to the overlying water, with from 40 to 290 g/hectare/day being released.

NITROGEN TRANSFORMATION PATHWAYS IN SEDIMENTS

The pathways involved in the cycling, gains and losses of nitrogen in sediment-water systems have been recently reviewed[7,8] (Fig. 2). In summary, the nitrogen cycle in sediment-water systems is largely microbial, and more complex than in most terrestrial systems. Several of the pathways are dominated by the oxidation-reduction (redox) status of the system, although evidence has been obtained in our laboratory of indirect effects due to pH, available carbonate-carbon and perhaps available calcium or phosphorus[23].

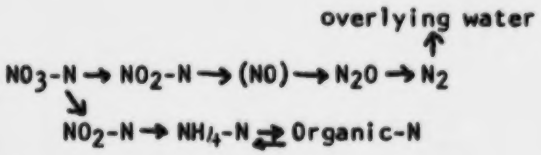
As stated preciously, the sediment is almost always in a highly reduced state. The only possible exception is in stirred shallow water sediments and at the sediment-water interface in oxygenated waters. To-date, we have not observed Eh values of >+100 mV in the sediment-water interface region, although locating the interface precisely is difficult if not impossible in most cases. This indicates that anoxic conditions prevail in the consolidated sediment, and that denitrification, N_2 fixation, and Fe and Mn reduction would be expected to occur. Below the interface, the Eh is negative, probably in the range of -200 to -300 mV for most sediments. Thus nitrogen transformations are carried out by facultative and obligate anaerobic bacteria.

Laboratory and field incubation studies using ammonium-^{15}N have shown that the ammonium-N in sediments is continually being assimilated while organic-N is being ammonified[12,15]. The net effect of the mineralization-immobilization reaction appears to be for ammonification to occur during the summer, and immobilization in the winter, in apparent response to sediment temperature[22].

Most sediments have the capacity to denitrify considerable nitrite-N and nitrate-N[21-26]. This may be of particular importance for lakes receiving nitrate-N in water entering the lake through the sediments by a hydraulically connected aquifer. Studies on a number of Wisconsin lakes indicate that over 60% of the nitrate-N added to sediments is denitrified, the remainder being immobilized[21,24,26]. Further, nitrate-N will be formed in oxygenated waters, and by diffusion or mixing, be carried into the sediment. No doubt most of this nitrate-N also will be lost from the system by denitrification. Some sediments, when stirred and oxygenated, are capable of rapid nitrification. It is plausible that nitrate-N could be formed in surficial sediments during high winds, with denitrification occurring during quiescent conditions[15,19]. This principle also could be used to remove nitrogen from lakes, using hypolimnion aeration combined with stratification to effect a nitrification-denitrification sequence.

Figure 2. The Nitrogen Cycle in Sediment-Water Systems[8].

Our denitrification research also has shown that considerable of the added nitrate-^{15}N is transformed to ammonium-N and organic-N. I had originally assumed this was entirely due to a mechanism involving preferential utilization of nitrate-N over ammonium-N by the sediment microbial population. However, even more recent work[26,27] indicates that N_2 fixation by anaerobes may be at least partially responsible for the internal cycling of nitrogen in sediments. The overall reaction sequence envisaged here is:



The net result of this sequence is that part of the nitrate-N that reaches sediments will be converted to available or potentially available nitrogen.

The potential for the addition of nitrogen to the lake ecosystem through N fixation in sediments was also evaluated in our laboratory[26,27]. The acetylene reduction method has been modified for sediment studies and applied to sediment samples from several Wisconsin lakes. Results obtained indicate that in the eutrophic, calcareous sediment lakes of southern Wisconsin, this pathway can add a significant amount of nitrogen to the lake[27].

SUGGESTED METHODS TO EVALUATE THE NITROGEN STATUS OF LAKE SEDIMENTS

The approach taken to this section is to provide recommendations for (a) the minimum of sampling and analysis of sediments required to obtain an estimate of their nitrogen status and (b) further process studies which would provide additional information, mainly for use in more detailed investigations. The methods given are those used currently in our laboratory. Many have been used extensively for research in soil N transformations, and have been modified as needed for use on sediments. Operating details of the methodology can be found in the references quoted.

SAMPLING

Obtaining a representative sample of lake sediment from most lakes may be one of the most critical steps in obtaining an estimate of sediment contribution to the nitrogen status of lakes, and the problem obviously is greater as the area of a lake increases. However, for survey purposes, detailed grid sampling of a lake is one approach. Alternatively, samples from the deepest portion of the lake as well as from intermediate and shallow water column depth could be obtained. Areas such as obviously isolated bays should be sampled independently. Profile water samples also should be taken at the time of sediment sampling.

Numerous sediment samplers are available, any of which might be preferable depending on sediment consistency and information desired. For relatively fluid (unconsolidated) sediments, the Eckman dredge works satisfactorily for grab samples, and simple, gravity driven stratification samplers (e.g., Soil Test Inc., Evanston, Illinois Catalog No. DR-1006) for cores of 20 to 30 cm depth. For consolidated sediments (e.g., sandy materials) a Peterson dredge, or more sophisticated corers[28,29] are necessary.

Core samples to at least the 20-cm depth are to be preferred even for routine survey work. However, dredge samples will suffice if a coring device and/or the man-power is not available. For laboratory work, a dredge sample usually is necessary due to the relatively large amount of sample required. If a dredge is to be used, the Eckman-type of dredge is preferred over a Peterson-type as the former obtains a more representative sample of the surficial sediments. At the time of sampling, free water should be decanted from the sediment sample.

The sediment (and water) samples must be handled in such a way as to minimize changes during transportation and storage. In particular, the sediment samples should be protected from exposure to the air, stored

at low temperatures (just above freezing) to minimize microbial activity and analyzed as soon as possible. We have found that plastic bottles permit diffusion of sufficient air that significant changes can occur, especially in smaller-sized samples. Large (10 to 20 liter) polyethylene containers are satisfactory for bulk samples. Sample bottles should be completely filled with sediment so that air is excluded. Water samples should be treated with phenyl mercuric acetate* and stored at 5° C. The sampling frequency is optional. We have noted significant periodic fluctuations of NH_4-N (soluble and exchangeable) in Lake Wingra sediments[22] with a maximum in the autumn and minimum in the spring. Ideally, sampling should be conducted weekly at overturn and every other week the rest of the time. At a minimum, sampling should be conducted at spring turnover, summer stratification (or about mid-August if the lake does not stratify), fall turnover, and mid-winter. If only one sampling time is possible, a relatively static period (i.e., mid-summer or mid-winter) would be the most feasible if only a comparison between lakes is desired.

On-Site Measurements

The following should be measured at the time of sampling: (a) water temperature profile (YSI or equivalent probe) to sediment-water interface; (b) dissolved oxygen profile to sediment water interface (YSI probe); (c) pH profile to sediment-water interface (may be taken later on water samples collected); (d) (optional) Eh profile to sediment-water interface (a platinum-black indicator electrode on a long lead, and calomel reference electrode dipped into the water surface provides a suitable electrode pair, (any portable Eh-pH meter with ± 700 mV range is satisfactory), and, (e) general limnological observations as desired (e.g., Secchi disk reading).

PHYSICAL CHARACTERISTICS OF THE SEDIMENT

Before subsampling, the sample should be mixed thoroughly. Subsampling can best be conducted by using a piston type coring device (about 1 cm diameter) or a hypodermic syringe with an opening enlarged sufficiently that it does not become clogged.

*Dissolve 0.1 g of phenyl mercuric acetate in 20 ml of dioxane. Dilute with water to 100 ml. Add 1 ml of this solution to each liter of water sample.

Sediment Water and Solids

Add a 100 ml sample to a tared 100-ml graduated beaker. Determine weight of wet sediment (w_{ws}). Evaporate the sediment to dryness on a hot plate at 60° C, and bring to constant weight (16-24 hrs) in an oven set at 105-110° C. Determine weight of oven-dry sediment (w_{ds}).

a) Sediment water content, ml/liter = $\frac{(w_{ws} - w_{ds})\ (10)}{dw}$ where dw = water at temperature of solutions.

b) Bulk density (D_b) = $\frac{w_{ws}}{100\ cc}$ in g/cc.

c) % Solids = 100 - % H_2O.

Extraction of Interstitial Water

Place 150 ml of sediment in a 200-ml polypropylene centrifuge bottle, centrifuge at 16,00 Xg (10,000 rpm for a 30 cm radius fixed head rotor Sorvall Superspeed RC-2 Automatic Refrigerated Centrifuge.) Decant the water and determine the water volume removed. Weigh the moist residue, allow the residue to air dry and determine the oven-dry weight of solids on a subsample. Calculate the amount of water retained by the sediment.

If the centrifuge used is different from the above, the relative centrifugal force can be calculated by:

$$g = (1.118 \times 10^{-5})\ (RPM)^2\ (\text{radius of rotation in cm, } R)$$

or:

$$RPM = \left[\frac{16{,}000\ Xg}{(1.118 \times 10^{-5})\ (R)} \right]$$

CHEMICAL CHARACTERIZATION OF SEDIMENTS

Presence of $CaCO_3$

Treat 10 ml of sediment with 5 ml of 1 N HCl and stir gently. If gas bubbles observed to evolve, the sediment is calcareous.

Carbon (optional)

Estimate organic C by determining the CO_2 evolved on combustion of the sediment sample at 650° C, and total C (if $CaCO_3$ is present) by CO_2 evolved on combustion at 900° C. Inorganic C = total C -organic C[30].

Report results to 3 significant figures as % of oven-dry sediment. Alternatively, inorganic C can be reported as $CaCO_3$:

$$\% CaCO_3 = (8.3)\ (\%\ \text{inorganic C})$$

Organic Matter Content

Estimate as the weight loss on ignition at 550° C for two hours of a one g sample of air-dry sediment. Correct results for water content of the air-dry sample, and report as % of oven-dry sediment.

Sulfur (optional)

Estimate total S by using the digestion methods for soil as described by Bardsley and Lancaster[31]. Estimate sulfide-S by distillation with external heat under N_2[31] of a sample treated with HCl, and inorganic forms of S (except sulfate) by distillation as above with the exception that mossy Zn is also added[32]. In the inorganic S procedures, sulfide is estimated as molybdenum blue.

Nitrogen Forms

Total Sediment Organic Nitrogen-

Either the semimicro or macro Kjeldahl procedure is satisfactory[33,34]. Total nitrogen can be determined on moist sediment or on the air-dry solids material. The values obtained should be corrected for inorganic forms present. Results can be expressed on the mg N per liter or % of oven-dry weight of solids basis.

Inorganic N-

Separate the interstitial water as described previously. Determine interstitial ammonium-N by steam distillation of a 10- to 50-ml aliquot of the water sample with 0.2 g of ignited MgO[35]. Subsequently, determine (nitrite plus nitrate)-N by steam distillation of the same aliquot with 0.2 g of finely divided Devarda Alloy[35]. Determine nitrite-N by the Gries-Illosvay method[34]. Extract the ammonium-N remaining on the sediment exchange sites (exchangeable ammonium-N) by treatment of the residue after centrifugation with 100 ml of 2 N KCl[18]. Determine ammonium-N in the extract by steam distillation with MgO. Express the results as mg of each form of nitrogen per liter of sediment. The exchangeable ammonium-N value must be corrected for the amount of interstitial ammonium-N remaining in the residue after centrifugation and for the increased volume due to water in this residue.

Soluble and Particulate Interstitial Water Nitrogen (optional)-

Determine total nitrogen (except nitrite-N and nitrate-N) on a 10- to 50-ml aliquot of interstitial water by the Kjeldahl procedure. Filter another aliquot through a 0.45 μ Millipore membrane filter, and determine organic N as above. Soluble organic-N = total-N of filtered sample minus interstitial water ammonium-N. Particulate-N equals total N of unfiltered sample minus soluble organic-N.

Forms of nitrogen in Lake Water-

Determine ammonium-N, nitrite-N and soluble organic N as described above. Nitrate-N is often below the detection limits of the distillation method and a more sensitive colorimetric method such as the one described by EPA[34] should be used.

PROCESS STUDIES

A few simple process studies are listed which, when performed as part of a lake survey, will give a relative indication of the biological activity and N release characteristics of the sediment-water system. For more detailed investigations, the reader should consult the papers listed or the author for recommendations.

Aerobic Nitrification

This test, while far from representative of in situ conditions, is suggested because it does seem to give a very simple index of potential biological activity in sediments[19]. Add 500 ml of sediment and 500 ml of water to a 2-liter widemouth jar. Add 10 g of reagent grade powdered $CaCO_3$ and a 1 ml suspension of a soil or sediment sample shown previously to be capable of nitrifying ammonium. Cover the bottle with a loosely-fitting cap of aluminum foil, and place in a dark, constant temperature cabinet at 10° C. Stir continuously using a magnetic stirrer and Teflon coated stirring bar. Remove 100-ml samples at 1, 3, 5, 7, 14 and 21-day intervals. Determine exchangeable and interstitial water ammonium-N and interstitial water nitrite-N and nitrate-N

The nitrate accumulation curve will have a delay phase of one to five days, and one or more linear phases. The first phase will correspond to the time when ammonium-N (exchangeable and soluble) is still present in the medium. During this time, ammonium-N is not limiting nitrifi-nitrification. A second phase will occur when the reaction: organic N → ammonium-N is rate-limiting. This rate will give an indication of the availability of sediment organic N.

Data should be presented as: (a) the delay phase (hours): (b) nitrification rate in the first linear phase (ug nitrogen/liter/hour).

Ammonium-N Formation Rate

Ammonium-N formation (net mineralization) under anaerobic conditions may prove to be a valuable index of nitrogen availability. This is more representative of *in situ* conditions than the aerobic nitrification test described previously, but is somewhat more difficult to perform because anaerobic conditions must be maintained. However, this can be done by sweeping the sample with helium before closing the flask, and using a tightly sealed system.

Place 100 ml of sediment in a 250-ml Erlenmeyer flask, fitted with a ground glass stopper that can be attached to the body of the flask by springs. Purge the atmosphere above the flask with helium, and stopper tightly. Set up sufficient samples so that duplicates can be analyzed at 0, 3, 7, 14, 28, 56 and 112 days. Incubate in the dark in a constant temperature cabinet at 10° C.

Express results as µg NH_4-N formed/liter sediment/day for each of the incubation periods (µg NH_4-N formed = incubated sediment value minus initial sediment value).

Nitrate Disappearance Rate

Chen et al.[21] noted significant differences in the rate of nitrate disappearance when nitrate was added to southern Wisconsin calcareous and northern Wisconsin noncalcareous sediments. Because nitrate-N can be lost from anaerobic systems via two pathways, namely denitrification nitrate-N → nitrogen gas and assimilatory nitrate reduction nitrate-N → ammonium-N and organic N), tracer studies with nitrogen-15 are needed to distinguish the relative rates of these pathways. However, the rate of nitrate disappearance should be an indication of the intensity and capacity of the redox buffering of the sediment, and will also integrate the rate of biological activity and organic matter availability.

To a 100 ml polyethylene centrifuge tube, add 50 ml of sediment and 5 ml of a 100 µg/ml nitrate-N (as KNO_3) solution. Purge the atmosphere with helium, stopper tightly, mix thoroughly, and place in a dark constant-temperature cabinet set at 10° C. Remove samples at approximately 30 minutes, 2 hours, 4 hours, 8 hours, 24 hours and 48 hours after incubation. (Shorter or longer incubation times may prove more feasible with particular samples.) Determine ammonium-N, nitrite-N and nitrate-N.

Express results as µg nitrate-N lost per liter of sediment per hour, and µg nitrite-N formed per liter of sediment per hour. (A plot of the results may prove helpful in interpretation.)

NH_4-N Exchange

This test will provide an indication of the amount of ammonium-N in solution and on the sediment exchange sites at varying levels of NH_4-N. Sediments that do not sorb as much ammonium-N on the exchange sites have the potential for releasing more ammonium-N to the overlying waters.

To a series of 25 ml samples of sediment in a 100 ml centrifuge tubes, add 25 ml of a solution containing 0, 10, 100, or 500 µg of ammonium-N per ml (as NH_4Cl). Purge the atmosphere with helium, stopper tightly and shake gently for four hr. Determine exchangeable and interstitial water ammonium-N.

Express results as % of added NH_4-N on the exchange sites and in the interstitial water, using zero ammonium-N addition as the control.

Effect of Sediment:Water Ratio on Ammonium-N Release

Dilution of sediments in the water, as might occur when a shallow sediment is stirred by wave action, currents, or mechanical action from fish, boats, gas bubbling, tends to favor release of ammonium from the exchange sites. This is because the equilibrium of the reaction:

$$X\text{-}NH_4 + \tfrac{1}{2}\ Ca^{++} \rightleftharpoons X\text{-}\tfrac{1}{2}\ Ca + NH_4^{+};\ X = \text{exchange material}$$

proceeds to the right as the concentration of cations in solution is decreased, which is the process which occurs on dilution. Comparison of the magnitude of the dilution effect between different sediments should indicate which sediments have the potential for contributing the most ammonium-N when disturbed.

To 20 ml of sediment in a 250 ml centrifuge tube, add 10, 20, 50, 100 or 200 ml water (preferably water from the lake sampled or alternatively, an artificial solution containing the salt concentration and hardness typical of the lakes being studied). Shake for four hours, and determine the ammonium in the interstitial and exchangeable fractions.

Express results as µg of ammonium-N per liter of sediment in each fraction with each treatment.

EVALUATION OF DATA OBTAINED

For the most part, the information and suggested parameters have been based on our experience. The methods are those we would utilize if given the charge of evaluating the nitrogen status of lakes on a modest budget. However, to date, insufficient observations on these parameters are available to adequately evaluate their usefulness or

establish numerical values to classify lakes with regard to the potential N contribution from sediments. The first task should be to correlate the suggested parameters with other information available, particularly trophic status of the lake using a large number of lakes of diverse types. From these data, one could then omit tests which did not give meaningful results.

The next phase would be to apply the tests which show promise over two- to three-years' duration on selected lakes. From these data, one should be able to devise a range of values for each test which would define closely the nitrogen status of the sediment.

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APPENDIX: LIST OF PROJECT PUBLICATIONS

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